CHANGES IN THE FUNGAL SUCCESSION AFTER APPLICATION OF UREA TO LITTER OF SCOTS PINE *(PINUS SYLVESTRIS* L.)

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In their description of the fungal succession on the needles of Scots Pine (*Pinus sylvestris* L.) in the Delamere Forest, England, KENDRICK and BURGES (1962) did not record the common primary saprophytes *Clasdosporium herbarum, Epicoccum purpurascens, Alternaria alternata* and *Botrytis cinerea* which HUDSON (1968) has described as important early colonisers of senescent leaves in temperate climates. As Hudson has pointed out, it is rare to find examples of decaying leaves where all the common saprophytes are not found (HUDSON, 1971), another example being the leaves of *Pseudoscleropodium purum* on which *B. cinerea* and *Aureobasidium pullulans* did not develop (KILBERTUS, 1968).

In a study of the fungi of Scots Pine needle-litter at Brandon Park, England (LEHMANN, 1973), the possibility of a nutritional basis for the absence of these saprophytes was investigated by treating the litter with fertilisers. $(NH_4)_2 SO_4$ at 10 g N/m² and KH_2PO_4 at 4,4 g P/m² were used but were found to have no appreciable effect on the pattern of fungal colonisation. Urea was also used as it had been shown to stimulate the growth of primary saprophytes on apple leaves (BURCHILL and COOK, 1971 ; HUDSON, 1971) and it brought about marked changes in the fungal succession on pine needles.

I. MATERIALS AND METHODS

Collection of needles and treatment for successional studies ;

Needles falling during six days in September 1972 were collected on nylon netting. They were individually tagged with a piece of aluminium bent about the fascicle end. After soaking in 8 % urea or in water for 1 hr, they were laid out on the litter surface in 2 x 1 m² quadrats. 8 % urea (10 g N/m²) was added as a spray to the treated quadrat, water being added to the control.

30 needles were sampled two weeks after treatment and then at monthly intervals, and the fungi recorded by three methods ; direct observation under a dissecting microscope, plating of 2 mm segments from surface-sterilised needles into 2 % malt extract agar containing streptomycin (0,2 mg/ml), and by spore fall (LEHMANN, 1973).

Treatment of plots with high levels of urea :

In January 1973, 1x1m² plots were treated with urea (20, 40 and 80 g N/m²) evenly applied in powder form. The macroscopic fruiting structures that developed were recorded during the following five months.

II. RESULTS — SUCCESSIONAL STUDIES

The predominant fungi developing on untreated pine needles are shown in Fig. 1. The virtual absence of common primary saprophytes is obvious. *Cladosporium herbarum* did occur but its development was restricted, typically only a few conidiophores being found on a needle. Of the main fungi, *Lophodermium pinastri* and *Lophodermium* B (= *L. pinastri* form B, MILLAR and WATSON, 1971) colonise the interior of living needles ; *Verticicladium trifidum, Fusicoccum bacillare* and the Basidiomycetes (including *Marasmius androsaceus)* colonise the interior of fallen needles ; *Lophiostoma pinastri* and *Sympodiella acicola* form a dark hyphal network on the needle surface ; *Sclerophoma pithyophila* colonises both the surface and the interior of the needles.

Needles that had been treated with 8 % urea (10 g N/m² plots) showed marked differences from the usual pattern of colonisation as shown in Fig. 1. The pattern of colonisation, shown by many typical pine needle fungi was changed and there was a stimulation of the growth of fungi unimportant or absent on control needles (Table 1).

Table 1

Changes in fungal colonisation of needles after treatment with 8 % urea (10 gN/m²) when compared to water control.

Fungi appearing, increasing or persisting longer :	
Sporobolomyces roseus	Polyscytalum fecundissimum
Cladosporium herbarum	Monilia sp.
Epicoccum purpurascens	Verticillium sp.
Phoma sp.	Lophodermium B (possibly)
Sclerophoma pithyophila	Trichoderma polysporum (possibly)
Fungi decreasing :	**No clear differences :**
Verticicladium trifidum	Lophodermium pinastri
White yeasts	Naemacyclus niveus
Basidiomycetes (incl.	Sympodiella acicola
Marasmius androsaceus)	Fusicoccum bacillare
Lophiostoma pinastri	

Figs. 2 and 3 show the pattern for the increased occurrence of some of the fungi. *Phoma* sp. and *Verticillium* sp. were absent on untreated needles but were common on those receiving urea. The growth of *Cladosporium herbarum* was greatly increased, a dense covering of conidiophores being visible two months after the urea treatment. Urea also brought about an increase in the occurrence of *Monilia* sp. and *Sclerophoma pithyophila* and a longer persistence of *Sporobolomyces roseus*.

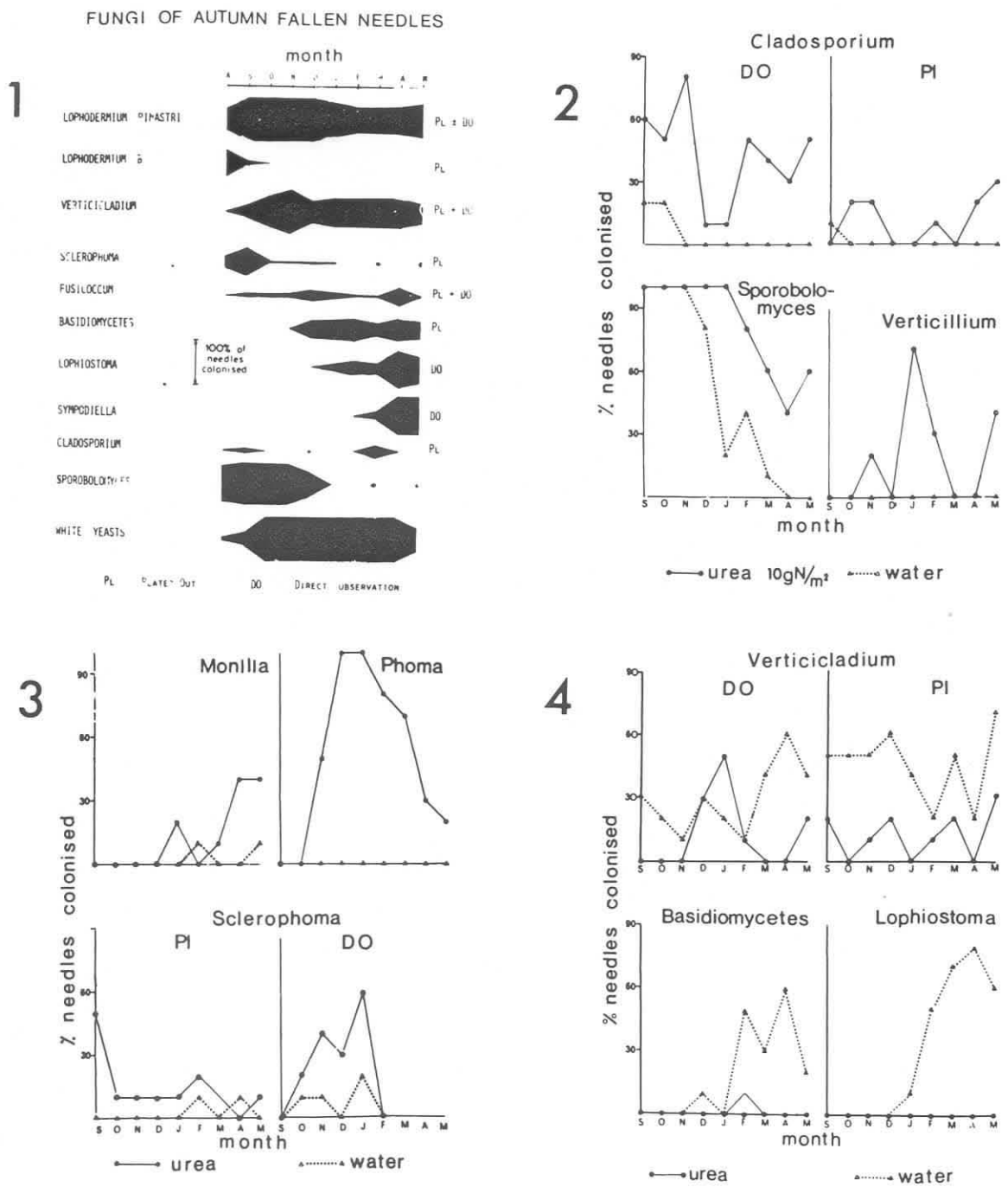

Fig. 1 : Schematic diagram showing the predominate fungi colonising untreated pine needles using the methods of direct observation (DO), plating out (PL), and spore fall. (From LEHMANN, 1973).

Fig. 2-4 : Effect of urea treatment on the colonisation of needles by *Cladosporium herbarum*, *Sporobolomyces roseus*, *Verticillium* sp., *Monilia* sp., *Phoma* sp., *Sclerophoma pithyophila*, *Verticicladium trifidum* and internally colonising Basidiomycetes and on the production of ascocarps by *Lophiostoma pinastri*.

The development of some fungi, inhibited by the urea treatment, is shown in Fig. 4. The colonisation by *Lophiostoma pinastri* was delayed and ascocarps did not form ; *Verticicladium trifidum* and the internally colonising basidiomycetes were strongly inhibited.

These changes in colonisation pattern were associated with changes in litter pH. The pH of urea treated needles was 5,5-6,0 one month after urea application, compared to pH 3,5-4,0 for untreated litter. In addition the litter darkened, this being unrelated to the growth of dark hyphae on the needle surface.

Urea treated plots

On the plots treated with urea (20, 40 and 80 g N/m²) in January 1973, there were some dramatic changes. The needles darkened and became waterlogged and various macroscopic fruiting structures appeared, which were not found on the control plots (Table 2).

Table 2

Occurrence of ascocarps or basidiocarps on plots treated with urea in January 1973 and recorded until May 1973.

	Months after application	Urea gN/m²			
		0	20	40	80
Marasmius androsaceus	0 - 5	+	—	—	—
Desmazierella acicola	4 - 5	+	—	—	—
Lophodermium pinastri	4 - 5	+	—	—	—
Ascobolus denudatus	1 - 2	—	+	+	+
Pseudombrophila deerata	1 - 3	—	+	+	—
Tephrocybe tesquorum	2 - 5	—	+	+	+
Coprinus echinosporus	3 - 4	—	+	+	+
Mycena sp.	2 - 4	—	+	+	+

\+ *Present*
— *Absent*

The fungi, which appeared solely on urea treated plots, increased in numbers with increasing amounts of urea, though in the case of *Pseudombophila deerata* (Fig. 5) there was no development of ascocarps at the highest level of urea applied.

Some of these fungi have been found on fertiliser treated litter by other workers ;

Tephrocybe tesquorum, (Fig. 6) which also grew where human urine had been applied, has been recorded, from litter treated with urea, ammonia, nitrogenous materials (including urine and dead animals) and alkalis (KOH, K_2CO_3 ; Na_2CO_3) by SAGARA and HAMADA (1965), HONGO and SAGARA (1967), PETERSEN (1970), HORA (1972) and SAGARA (1973). It has also been associated with decaying agaric fruit bodies (LANGE and HORA, 1963), several of which are known to contain urea or urea producing compounds (TYLER, BENEDICT and STUNTZ, 1965).

Coprinus echinosporus (Fig. 7) has been recorded from plots treated with alkali (PETERSEN, 1970 ; HORA, 1972).

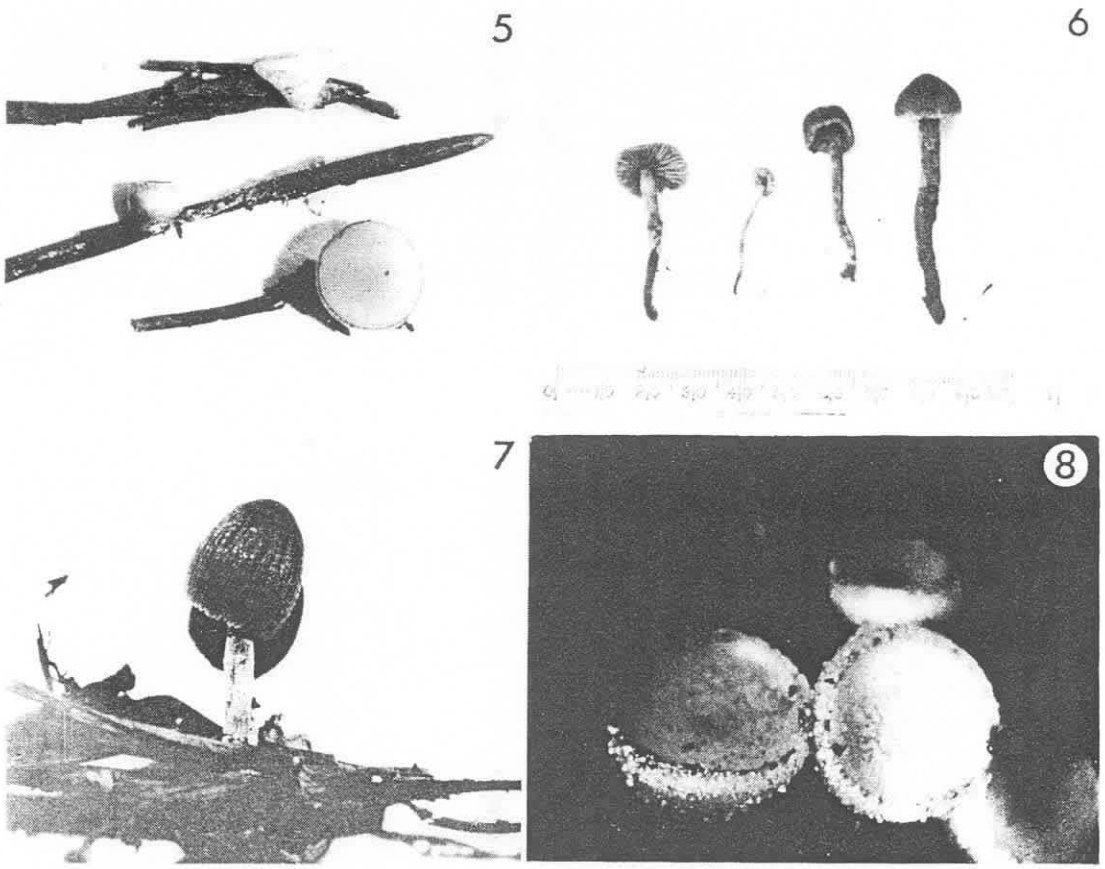

Fig. 5 : *Pseudombrophila deerata*.
Fig. 6 : *Tephrocybe tesquorum*.
Fig. 7 : *Coprinus echinosporus*.
Fig. 8 : *Ascobolus denudatus*.

Ascobolus denudatus (Fig. 8), which also grew where urine had been applied, has been recorded on litter treated with urea and related nitrogenous materials and with alkalis (KORF, 1965 ; PETERSEN, 1970).

III. DISCUSSION

The fungal succession on untreated needles was similar to that described by other workers. The absence of common primary saprophytes was obvious, and it is of interest that addition of urea allowed colonisation by some of these *(Cladosporium herbarum* and *Epicoccum purpurascens)*. In this way, the changes found, after a urea application to pine needles, parallels what has been found with urea treated apple leaves, where a large

increase in common primary saprophytes was seen and *Phoma* sp. colonised (BURCHILL and COOK, 1971 ; HUDSON, 1971).

Higher doses of urea allowed other fungi to develop (Table 2). The lack of a similar effect of $(NH_4)_2SO_4$ indicates that the effect of urea is not solely that of an N-fertiliser. It may be argued that $(NH_4)_2SO_4$ would be rapidly leached from litter, but far higher levels than 10 g N/m^2 have been used (HORA, 1972 ; SAGARA, 1973) and have not produced similar changes.

The ability of urea to produce alkaline conditions, seems an important part of its activity. Both urea and alkalis bring about increasing pH and water soaking of litter and several fungi have been reported as common to alkali and urea treated plots (Table 3).

Table 3

The occurrence of fungi on fertiliser treated plots, Data in LEHMANN (1973).

	Treatment		
	Urea	Alkali	Lime and $CaCO_3$
Laccaria proxima	+	+	+
Peziza sp. 1	+	+	+
Ascobolus denudatus	+	+	—
Ascobolus sp. n° 2	+	+	—
Coprinus echinosporus	+	+	—
C. insignis	+	+	—
Fimaria (?) sp.	+	+	—
Gelatinodiscus sp.	+	+	—
Hebeloma vinosophyllum	+	+	—
Tephrocybe ambusta	+	+	—
T. tesquorum	+	+	—
Clitocybe metachroa	—	+	+
Neuronectria peziza	—	+	+
Rhizopogon rubescens	+	—	+

\+ *Recorded*
— *Not recorded*

Table 3 also shows that lime does not act in the same way as urea and alkalis as there are few fungi in common. SAGARA (1973) has suggested that urea and the alkalis act by inducing NH_3 release in the litter, something that is also released by protein containing materials. Ammonia release might inhibit the growth of the usual pine needle colonisers and might lower their capability of producing antibiotics, so allowing colonisation by the common primary saprophytes.

Whilst the exact mechanism of action of urea is not known, we may expect that similarity of the effects of urea, alkali and protein will be found with the microfungi, which have been little studied. Indeed, rust infected leaves support a higher number of several

common primary saprophytes than uninfected leaves (E.-H.-C. MCKENZIE — this colloquium). Possibly decaying rust mycelium supports the growth of the common primary saprophytes in a similar way as decaying agarics support *Tephrocybe tesquorum*.

Thus the addition of urea to pine needle litter changes the pattern of fungal colonisation and allows fungi, normally absent from pine needles, to colonise. Elucidation of the exact mechanism may provide an explanation for the unimportance of the common primary saprophytes on pine needles.

IV. Acknowledgements

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